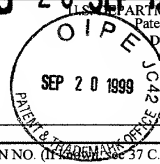


520 Rec'd PCT/PTO

20 SEP 1999

097381598

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. § 371



U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office
Docket No: 350292000800

U.S. APPLICATION NO. (If Applicable) 37 C.F.R. § 1.5: (unassigned)

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/JP98/01217

20 March 1998

21 March 1997

TITLE OF INVENTION: **A PREVENTIVE OR THERAPEUTIC AGENT FOR SENSITIZED T CELL-MEDIATED DISEASES COMPRISING IL-6 ANTAGONIST AS AN ACTIVE INGREDIENT**

APPLICANTS FOR DO/EO/US: Masahiko MIHARA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.
3. This express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☒ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☒ An executed oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A Notification of the Recording of a Change letter.
16. ☒ Other items or information: 1) 5 pages of International Form of Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure; 2) PCT Information Concerning Elected Offices Notified of Their Election; 3) PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices; 4) a return postcard.


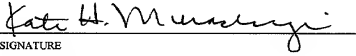
CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Express Mail Label No.: EJ004416834US

Date of Deposit: September 20, 1999

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Sheri N. Shippe
Sheri N. Shippe

U.S. APPLICATION NO. (If known, see 37 C.F.R. § 1.51) (unassigned) <div style="font-size: 1.5em; font-weight: bold; margin-left: 100px;">09/381598</div>	INTERNATIONAL APPLICATION NO. PCT/JP98/01217	DOCKET NUMBER: 350292000800
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO\$840.00 International preliminary examination fee paid to (USPTO (37 C.F.R. § 1.482))\$670.00 No international preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) but international search fee paid to USPTO (37 C.F.R. § 1.445(a)(2))\$760.00 Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO\$970.00 International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$96.00		CALCULATIONS PTO USE ONLY <div style="text-align: center; margin-top: 20px;">  </div>
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 840
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).		\$
CLAIMS	NUMBER FILED	NUMBER EXTRA
Total claims	22 - 20 =	2
Independent claims	3 - 3 =	0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		+ \$260.00
TOTAL OF ABOVE CALCULATIONS =		\$1,136
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 C.F.R. §§ 1.9, 1.27, 1.28)		
SUBTOTAL =		\$1,136
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)).		+
TOTAL NATIONAL FEE =		\$1,136
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). \$40 per property		+
TOTAL FEES ENCLOSED =		\$1,176
Amount to be refunded:		\$
charged:		\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ 1,176.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 03-1952 in the amount of \$0.00 to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Assistant Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 03-1952 . NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: Morrison & Foerster LLP 2000 Pennsylvania Avenue, N.W. Washington, D.C. 20006-1888 Telephone: (202) 887-1533 Facsimile: (202) 887-0763		
<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: left;">  SIGNATURE </div> <div style="text-align: right;"> Kate H. Murashige Registration No. 29,959 </div> </div>		



11/PRTS

514 Rec'd PCT/PTO

09/381598
20 SEP 1999
CGI-E910/PCT

- 1 -

DESCRIPTION

PREVENTIVE OR THERAPEUTIC AGENT FOR SENSITIZED T CELL-MEDIATED DISEASES COMPRISING IL-6 ANTAGONIST AS AN ACTIVE
INGREDIENT

Technical Field

The present invention relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising an interleukin-6 (IL-6) antagonist as an active ingredient. The present invention also relates to an inhibitor of sensitized T cells comprising an interleukin-6 (IL-6) antagonist as an active ingredient. Furthermore, the present invention relates to a suppressive agent of sensitized T cells comprising an antibody directed against IL-6 receptor as an active ingredient.

Background Art

IL-6 is a cytokine which is also called B cell stimulating factor 2 (BSF2) or interferon β 2. IL-6 was discovered as a differentiation factor involved in the activation of B-lymphatic cells (Hirano, T. et al., Nature (1986) 324, 73-76). Thereafter, it was found to be a multifunctional cytokine that influences various functions of cells (Akira, S. et al., Adv. in Immunology (1993) 54, 1-78). IL-6 has been reported to induce the maturation of T-lymphatic cells (Lotz et al., J. Exp. Immunol. 18: 1253-1258, 1988).

IL-6 transmits its biological activity through two types of proteins on the cell. One of them is IL-6 receptor, a ligand-binding protein with a molecular weight of about 80 kD, to which IL-6 binds. IL-6 receptor occurs not only in a membrane-bound form that penetrates through and is expressed on the cell membrane but also as a soluble IL-6 receptor consisting mainly of the extracellular region.

The other protein is a membrane-bound protein gp130

having a molecular weight of about 130 kD that is involved in signal transduction. IL-6 and IL-6 receptor form the IL-6/IL-6 receptor complex which, after binding to gp130, transmits its biological activity to the cell (Taga, T. et al., J. Exp. Med. (1987) 166, 967).

IL-6 antagonist is a substance that inhibits the transduction of biological activity of IL-6. As the IL-6 antagonist, there have been known so far antibody directed against IL-6 (anti-IL-6 antibody), antibody directed against IL-6 receptor (anti-IL-6 receptor antibody), and antibody directed against gp130 (anti-gp130 antibody). In addition, there are also known IL-6 antagonists that are disclosed in the International Patent Application WO 95-00852, the International Patent Application WO 95-11303, the International Patent Application WO 96-34104, the International Patent Application WO 96-18648, the International Patent Application WO 96-17869, Japanese Unexamined Patent Publication (Kokai) No. 7(1995)-324097, and Japanese Unexamined Patent Publication (Kokai) No. 8(1996)-311098.

Anti-IL-6 receptor antibody has been described in several reports (Novick D. et al., Hybridoma (1991) 10, 137-146, Huang, Y. W. et al., Hybridoma (1993) 12, 621-630, International Patent Application WO 95-09873, French Patent Application FR 2694767, United States Patent US 521628). A humanized PM-1 antibody was obtained by grafting the complementarity determining regions (CDRs) of a mouse antibody PM-1 (Hirata et al., J. Immunology (1989) 143, 2900-2906), to a human antibody (the International Patent Application WO 92-19759).

On the other hand, in many autoimmune diseases and allergic diseases, there are T cells that recognize specific antigens (sensitized T cells) and these sensitized T cells are known to be involved in the pathology of such diseases. For example, there are known the presence of sensitized T cells that are directed to myelin basic protein in multiple sclerosis (Zhang, J. et

al., J. Exp. Med (1994) 179, 973-984), S antigen in
uveitis (Nussenblatt, R. B. et al., Am. J. Ophthalmol
(1980) 89, 173-179), thyroglobulin in chronic
thyroiditis, foods and acarids for atopic dermatitis
5 (Kubota, Y. et al., J. Dermatol (1993) 20, 85-87, Kondo,
N. et al., J. Allergy Clin. Immunol (1993) 91, 658-668),
bacteria, viruses, fungi, etc. in delayed
hypersensitivity, and metal, Japanese lacquer, etc. in
contact dermatitis, and the like.

10 Furthermore, it is also possible to induce
pathological states similar to those in humans by
immunizing an animal with these antigens or by
introducing antigen-specific sensitized T cells into a
non-immunized animal. Based on these facts, it is
15 thought that sensitized T cells play an important role in
the above diseases. Currently, steroids and/or
immunosuppressive agents are used for the treatment of
these diseases, but they are symptomatic treatments and
require administration for a long period of time, which
20 eventually poses the problem of side effects.

It has not been known so far that IL-6 antagonists
as described above exhibit a suppressive effect on
sensitized T cells and a therapeutic effect on the
diseases in which sensitized T cells are involved.

25 Disclosure of the Invention

It is an object of the present invention to provide
a therapeutic agent for sensitized T cell-mediated
diseases said agent being free of the above-mentioned
30 drawbacks.

Thus, the present invention relates to a preventive
or therapeutic agent for sensitized T cell-mediated
diseases comprising an IL-6 antagonist as an active
ingredient.

35 The present invention also relates to a preventive
or therapeutic agent for sensitized T cell-mediated
diseases comprising an antibody directed against IL-6

receptor as an active ingredient.

5 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising a monoclonal antibody directed against IL-6 receptor as an active ingredient.

10 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising a monoclonal antibody directed against human IL-6 receptor as an active ingredient.

15 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising a monoclonal antibody directed against mouse IL-6 receptor as an active ingredient.

20 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising PM-1 antibody as an active ingredient.

25 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising MR16-1 antibody as an active ingredient.

30 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising an antibody directed against IL-6 receptor having the constant region (C region) of human antibody as an active ingredient.

35 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising a chimeric antibody or a humanized antibody directed against IL-6 receptor as an active ingredient.

 The present invention also relates to a preventive or therapeutic agent for sensitized T cell-mediated diseases comprising humanized PM-1 antibody as an active ingredient.

 The present invention also relates to a preventive or therapeutic agent for multiple sclerosis, uveitis,

chronic thyroiditis, delayed hypersensitivity, contact dermatitis, or atopic dermatitis comprising the above IL-6 antagonist as an active ingredient.

5 The present invention also relates to an suppressive agent of sensitized T cells comprising IL-6 antagonist as an active ingredient.

The present invention also relates to an suppressive agent of sensitized T cells comprising an antibody directed against IL-6 receptor as an active ingredient.

10

Brief Explanation of Drawings

Fig. 1 shows a suppressive action on the mouse delayed-type foot pad edema reaction by MR16-1 after the simultaneous administration of MR16-1 as the sensitization by tubercle bacillus.

15

Embodiment for Carrying Out the Invention

1. IL-6 antagonist

20 IL-6 antagonists for use in the present invention may be of any origin, any kind, and any form, as long as they have a suppressive effect on sensitized T cells, a preventive or therapeutic effect for diseases in which sensitized T cells are involved.

25 IL-6 antagonists block signal transduction by IL-6 and inhibit the biological activity of IL-6. As the IL-6 antagonist, there can be mentioned anti-IL-6 antibody, anti-IL-6 receptor antibody, anti-gp130 antibody, altered IL-6, or partial peptides of IL-6 or IL-6 receptor.

30 1-1. Anti-IL-6 antibody

Anti-IL-6 antibodies for use in the present invention can be obtained as polyclonal or monoclonal antibodies using a known method. As the anti-IL-6 antibodies for use in the present invention, monoclonal antibodies of, in particular, a mammalian origin, are preferred. Monoclonal antibodies of a mammalian origin include those produced by a hybridoma and recombinant

35

antibody produced by a host which has been transformed with an expression vector containing genetically engineered antibody genes. These antibodies, via binding to IL-6, inhibit the binding of IL-6 to IL-6 receptor, and thereby blocks signal transduction of the biological activity of IL-6 into the cell.

Examples of such antibodies include MH166 (Matsuda et al., Eur. J. Immunol. (1988) 18, 951-956) and SK2 antibody (Sato, K. et al., The 21st Nihon Mennekigakkai Soukai (General Meeting of the Japan Immunology Society), Academic Record (1991) 21, 166) and the like.

1-1-1. Preparation of IL-6

An anti-IL-6 antibody-producing hybridoma can be basically constructed using a known procedure as described below. Thus, IL-6 may be used as a sensitizing antigen and is immunized in the conventional method of immunization. The immune cells thus obtained are fused with known parent cells in the conventional cell fusion process, and then monoclonal antibody-producing cells are screened by the conventional screening method to prepare the desired hybridoma.

Specifically, anti-IL-6 antibody may be obtained in the following manner. For example, a human antigen used as the sensitizing antigen can be obtained using the IL-6 gene sequence/amino acid sequence disclosed in Eur. J. Biochem (1987) 168, 543, J. Immunol. (1988) 140, 1534, or Argic. Biol. (1990) 54, 2685.

After a suitable host cell was transformed by inserting the IL-6 gene sequence into a known expression vector system, the IL-6 protein of interest is purified from the host cell or the culture supernatant thereof. The purified IL-6 protein can be used as a sensitizing antigen. Alternatively, a fusion protein of the IL-6 protein and another protein may be used as a sensitizing antigen.

1-2. Anti-IL-6 receptor antibody

Anti-IL-6 receptor antibodies for use in the present invention can be obtained as polyclonal or monoclonal antibodies using a known method. As the anti-IL-6 antibodies for use in the present invention, 5 monoclonal antibodies of, in particular, a mammalian origin, are preferred. Monoclonal antibodies of a mammalian origin include those produced by a hybridoma and those produced by a host which has been transformed with an expression vector containing genetically 10 engineered antibody genes. The antibodies, via binding to IL-6 receptor, inhibit the binding of IL-6 to IL-6 receptor, and thereby block the transduction of the biological activity of IL-6 into the cell.

Examples of such antibodies include MR16-1 15 antibody (Saito, et al., J. Immunology (1993) 147, 168-173), PM-1 antibody (Hirata, et al., J. Immunology (1989) 143, 2900-2906), or AUK12-20 antibody, AUK64-7 antibody or AUK146-15 antibody (International Patent Application WO 92-19759), and the like. Among them, PM-1 antibody is 20 most preferred.

Incidentally, the hybridoma cell line which produces PM-1 antibody has been internationally deposited under the provisions of the Budapest Treaty as PM-1 on July 10, 1990 with the National Institute of Bioscience and Human Technology, Agency of Industrial Science and 25 Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, as FERM BP-2998. And the hybridoma cell line which produces MR16-1 antibody has been internationally deposited under the provisions of the Budapest Treaty as Rat-mouse hybridoma MR16-1 on March 30 13, 1997 with the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, as FERM BP-5875.

35 1-2-1. Preparation of IL-6 receptor

Hybridomas producing a monoclonal antibody can be basically prepared using a known procedure as

described bellow. Thus, IL-6 receptor is used as a sensitizing antigen and is immunized according to the conventional method of immunization. The immune cells thus obtained are fused with known parent cells in the conventional cell fusion process, and then monoclonal antibody-producing cells may be screened by the conventional screening method to prepare the desired hybridoma.

Specifically, anti-IL-6 receptor antibody may be prepared in the following manner. For example, the human IL-6 receptor used as the sensitizing antigen for obtaining antibody can be obtained using the IL-6 receptor gene sequence/amino acid sequence disclosed in European Patent Application EP 325474, and the mouse IL-6 receptor can be obtained using that disclosed in Japanese Unexamined Patent Publication (Kokai) 3(1991)-155795.

There are two types of IL-6 receptor proteins: IL-6 receptor expressed on the cell membrane, and IL-6 receptor detached from the cell membrane (soluble IL-6 Receptor) (Yasukawa et al., J. Biochem. (1990) 108, 673-676). Soluble IL-6 receptor antibody is composed substantially of the extracellular region of the IL-6 receptor bound to the cell membrane, and thereby is different from the membrane-bound IL-6 receptor in that the latter lacks the transmembrane region or both of the transmembrane region and the intracellular region.

After the gene sequence of IL-6 receptor was inserted into a known expression vector system to transform an appropriate host cell, the desired IL-6 receptor protein may be purified from the host cell or a culture supernatant thereof using a known method. The IL-6 receptor protein thus purified may be used as the sensitizing antigen. Alternatively, cells that are expressing IL-6 receptor protein or a fusion protein of the IL-6 receptor protein and another protein may be used as the sensitizing antigen.

E. coli that has a plasmid pIBBSF2R containing

cdna encoding human IL-6 receptor has been internationally deposited under the provisions of the Budapest Treaty as HB101-pIBIBSF2R on January 9, 1989 with the National Institute of Bioscience and Human
5 Technology, Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, as FERM BP-2232.

1-3. Anti-gp130 antibody

Anti-gp130 antibodies for use in the present
10 invention can be obtained as polyclonal or monoclonal antibodies using a known method. As the anti-gp130 antibodies for use in the present invention, monoclonal antibodies of, in particular, a mammalian origin, are preferred. Monoclonal antibodies of a mammalian origin
15 include those produced by a hybridoma and those produced by a host which has been transformed with an expression vector containing genetically engineered antibody genes. The antibodies, via binding to gp130, inhibit the binding of IL-6/IL-6 receptor complex to gp130, and thereby block
20 the transduction of the biological activity of IL-6 into the cell.

Examples of such antibodies include AM64 antibody (Japanese Unexamined Patent Publication (Kokai) 3(1991)-219894), 4B11 antibody and 2H4 antibody
25 (US5571513), B-S12 antibody and B-P8 antibody (Japanese Unexamined Patent Publication (Kokai) 8(1996)-291199).

1-3-1. Preparation of gp130

A monoclonal antibody-producing hybridoma can be basically constructed using a known procedure as
30 described below. Thus, gp130 may be used as a sensitizing antigen and is immunized in the conventional method of immunization. The immune cells thus obtained are fused with known parent cells in the conventional cell fusion process, and then the monoclonal antibody-
35 producing hybridomas are screened by the conventional screening method to prepare the desired hybridoma.

Specifically, monoclonal antibody may be

obtained in the following manner. For example, gp130 used as the sensitizing antigen can be obtained using the IL-6 receptor gene sequence/amino acid sequence disclosed in European Patent Application EP 411946.

5 After a suitable host cell was transformed by inserting the gp130 gene sequence into a known expression vector system, the gp130 protein of interest is purified from the host cell or from the culture supernatant thereof. The purified gp130 protein can be used as the
10 sensitizing antigen. Alternatively, cells that are expressing IL-6 receptor protein or a fusion protein of the gp130 protein and another protein may be used as the sensitizing antigen.

1-4. Preparation of antibody-producing hybridoma
15 Though mammals to be immunized with the sensitizing antigen are not specifically limited, they are preferably selected in consideration of their compatibility with the parent cell for use in cell fusion. They generally include, but not limited to,
20 rodents such as mice, rats, hamsters and the like.

Immunization of animals with a sensitizing antigen is carried out using a known method. A general method, for example, involves the intraperitoneal or subcutaneous administration of a sensitizing antigen to
25 the mammal. Specifically, a sensitizing antigen which has been diluted and suspended in an appropriate amount of phosphate buffered saline (PBS) or physiological saline etc. is mixed, as desired, with an appropriate amount of a common adjuvant, for example Freund's
30 complete adjuvant. After being emulsified, it is preferably administered to a mammal for several times every 4 to 21 days. Alternatively a suitable carrier may be used at the time of immunization of the sensitizing antigen.

35 After immunization and the confirmation of the increase in the desired antibody levels in the serum, the immune cells are taken out from the mammal and are

subjected to cell fusion, in which preferred immune cells include, in particular, the spleen cells.

The mammalian myeloma cells as the other parent cells which are subjected to cell fusion with the above-mentioned immune cells preferably include various known cell lines such as P3X63Ag8.653) (J. Immunol. (1979) 123: 1548-1550), P3X63Ag8U.1 (Current Topics in Microbiology and Immunology (1978) 81: 1-7), NS-1 (Kohler, G. and Milstein, C., Eur. J. Immunol. (1976) 6: 511-519), MPC-11 (Margulies, D.H. et al., Cell (1976) 8: 405-415), SP2/0 (Shulman, M. et al., Nature (1978) 276: 269-270), FO (de St. Groth, S. F. et al., J. Immunol. Methods (1980) 35: 1-21), S194 (Trowbridge, I.S., J. Exp. Med. (1978) 148: 313-323), R210 (Galfre, G. et al., Nature (1979) 277: 131-133) and the like.

Cell fusion between the above immune cells and the myeloma cells may be essentially conducted in accordance with a known method such as is described in Milstein et al. (Kohler, G. and Milstein, C., Methods Enzymol. (1981) 73: 3-46) and the like.

More specifically, the above cell fusion is carried out in the conventional nutrient broth in the presence of, for example, a cell fusion accelerator. As the cell fusion accelerator, for example, polyethylene glycol (PEG), Sendai virus (HVJ) and the like may be used, and, in addition, an adjuvant such as dimethyl sulfoxide etc. may be added as desired to enhance efficiency of the fusion.

The preferred ratio of the immune cells and the myeloma cells to be used is, for example, 1 to 10 times more immune cells than the myeloma cells. Examples of culture media to be used for the above cell fusion include RPMI1640 medium and MEM culture medium suitable for the growth of the above myeloma cell lines, and the conventional culture medium used for this type of cell culture, and besides a serum supplement such as fetal calf serum (FCS) may be added.

In cell fusion, predetermined amounts of the above immune cells and the myeloma cells are mixed well in the above culture liquid, to which a PEG solution previously heated to about 37 °C, for example a PEG
5 solution with a mean molecular weight of about 1000 to 6000, is added at a concentration of 30 to 60% (w/v) and mixed to obtain the desired fusion cells (hybridomas). Then by repeating the sequential addition of a suitable culture liquid and centrifugation to remove the
10 supernatant, cell fusion agents etc., which are undesirable for the growth of the hybridoma, can be removed.

Said hybridoma is selected by culturing in the conventional selection medium, for example, the HAT
15 culture medium (a culture liquid containing hypoxanthine, aminopterin, and thymidine). Culturing in said HAT culture medium is continued generally for a period of time sufficient to effect killing of the cells other than the desired hybridoma (non-fusion cells), generally
20 several days to several weeks. The conventional limiting dilution method is conducted in which the hybridomas that produce the desired antibody are screened and monclonally cloned.

In addition to obtaining the above hybridoma by
25 immunizing an animal other than the human with an antigen, it is also possible to sensitize human lymphocytes in vitro with desired antigen or desired antigen-expressing cells, and the resulting sensitized B lymphocytes are fused with a human myeloma cell, for
30 example U266, to obtain the desired human antibody having the activity of binding to desired antigen or desired antigen-expressing cells (see Japanese Post-examined Patent Publication (Kokoku) No. 1(1989)-59878). Furthermore, a transgenic animal having a repertoire of
35 all human antibody genes is immunized with the antigen or the antigen-expressing cells to obtain the desired human antibody in the method described above (see International

Patent Application WO 93/12227, WO 92/03918, WO 94/02602, WO 94/25585, WO 96/34096 and WO 96/33735).

5 The monoclonal antibody-producing hybridomas thus constructed can be subcultured in the conventional culture liquid, or can be stored for a prolonged period of time in liquid nitrogen.

10 In order to obtain monoclonal antibodies from said hybridoma, there can be mentioned a method in which said hybridoma is cultured in the conventional method and the antibodies are obtained as the supernatant, or a method in which the hybridoma is administered to and grown in a mammal compatible with said hybridoma and the antibodies are obtained as the ascites. The former method is suitable for obtaining high-purity antibodies, 15 whereas the latter is suitable for a large scale production of antibodies.

Specifically the hybridoma producing anti-IL-6 receptor antibody can be constructed using the method disclosed in Japanese Unexamined Patent Publication 20 (Kokai) 3(1989)-139293. It can be conducted by a method in which the PM-1 antibody-producing hybridoma that was internationally deposited under the provisions of the Budapest Treaty as FERM BP-2998 on July 10, 1990 with the National Institute of Bioscience and Human Technology, 25 Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, is intraperitoneally injected to BALB/c mice (manufactured by CLEA Japan) to obtain the ascites from which the PM-1 antibody is purified, or: a method in which said 30 hybridoma is cultured in a suitable culture medium such as the RPMI1640 medium containing 10% bovine fetal serum and 5% BM-Condimed H1 (manufactured by Boehringer Mannheim), the hybridoma SFM medium (manufactured by GIBCO-BRL), the PFHM-II medium (manufactured by GIBCO-BRL) and the like, and the PM-1 antibody can be purified 35 from the supernatant.

1-5. Recombinant antibody

A recombinant antibody which was produced by the recombinant gene technology in which an antibody gene was cloned from the hybridoma and integrated into a suitable vector which was then introduced into a host can be used in the present invention as monoclonal antibody (see, for example, Carl, A.K., Borrebaeck, and James, W. Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, published in the United Kingdom by MACMILLAN PUBLISHERS LTD. 1990).

Specifically, mRNA encoding the variable (V) region of the desired antibody is isolated from the hybridoma producing the antibody. The isolation of mRNA is conducted by preparing total RNA using, for example, a known method such as the guanidine ultracentrifuge method (Chirgwin, J.M. et al., Biochemistry (1979) 18, 5294-5299), the AGPC method (Chmczynski, P. et al., (1987) 162, 156-159), and then mRNA is purified from the total RNA using the mRNA Purification kit (manufactured by Pharmacia) and the like. Alternatively, mRNA can be directly prepared using the Quick Prep mRNA Purification Kit (manufactured by Pharmacia).

cDNA of the V region of antibody may be synthesized from the mRNA thus obtained using a reverse transcriptase. cDNA may be synthesized using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit and the like. Alternatively, for the synthesis and amplification of cDNA, the 5'-Ampli FINDER RACE Kit (manufactured by Clontech) and the 5'-RACE method (Frohman, M.A. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 8998-9002; Belyavsky, A. et al., Nucleic Acids Res. (1989) 17, 2919-2932) that employs polymerase chain reaction (PCR) may be used. The desired DNA fragment is purified from the PCR product obtained and may be ligated to vector DNA. Moreover, a recombinant vector is constructed therefrom and then is introduced into E. coli etc., from which colonies are selected to prepare the desired recombinant vector. The base sequence of the desired DNA may be confirmed by a known method such as

the dideoxy method.

Once the DNA encoding the V region of the desired antibody has been obtained, it may be ligated to DNA encoding the constant region (C region) of the desired antibody, which is then integrated into an expression vector. Alternatively, the DNA encoding the V region of the antibody may be integrated into an expression vector which already contains DNA encoding the C region of the antibody.

In order to produce the antibody for use in the present invention, the antibody gene is integrated as described below into an expression vector so as to be expressed under the control of the expression regulatory region, for example an enhancer and/or a promoter. Subsequently, the expression vector may be transformed into a host cell and the antibody can then be expressed therein.

1-6. Altered antibody

In accordance with the present invention, artificially altered recombinant antibody such as chimeric antibody and humanized antibody can be used for the purpose of lowering heterologous antigenicity against humans. These altered antibody can be produced using known methods.

Chimeric antibody can be obtained by ligating the thus obtained DNA encoding the V region of antibody to DNA encoding the C region of human antibody, which is then integrated into an expression vector and introduced into a host for production of the antibody therein (see European Patent Application EP 125023, and International Patent Application WO 96/02576). Using this known method, chimeric antibody useful for the present invention can be obtained.

For example, the plasmid that contains DNA encoding the L chain V region or the H chain V region of chimeric PM-1 antibody was designated as pPM-k3 or pPM-h1, respectively, and E. coli having the plasmid has been

internationally deposited under the provisions of the Budapest Treaty as NCIMB 40366 and NCIMB 40362, respectively, on February 11, 1991 with the National Collections of Industrial and Marine Bacteria Limited (see International Patent Application WO 92-19759).

Humanized antibody which is also called reshaped human antibody has been made by grafting the complementarity determining regions (CDRs) of antibody of a mammal other than the human, for example mouse antibody, into the CDRs of a human antibody. The general recombinant DNA technology for preparation of such antibodies is also known (see European Patent Application EP 125023 and International Patent Application WO 96/02576).

Specifically, a DNA sequence which was designed to ligate the CDR of mouse antibody with the framework region (FR) of human antibody is synthesized from several divided oligonucleotides having sections overlapping with one another at the ends thereof by the PCR technique.

The DNA thus obtained is ligated to the DNA encoding the C region of human antibody and then is integrated into an expression vector, which is introduced into a host for antibody production (see European Patent Application EP 239400 and International Patent Application WO 92-19759).

For the FR of human antibody ligated through CDR, the complementarity determining region that forms a favorable antigen binding site is selected. When desired, amino acids in the framework region of the antibody variable region may be substituted so that the complementarity determining region of reshaped human antibody may form an appropriate antigen binding site (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

For chimeric antibody or humanized antibody, the C region of human antibody is used. As the C region of human antibody, there can be mentioned Cy, and Cy1, Cy2, Cy3, and Cy4, for example, can be used. The C

region of human antibody may be modified to improve the stability of antibody or the production thereof.

Chimeric antibody consists of the variable region of antibody derived from a mammal other than the human and the C region derived from human antibody, whereas humanized antibody consists of the complementarity determining region of antibody derived from a mammal other than the human and the framework region (FR) and the C region of antibody derived from human antibody. Accordingly, antigenicity thereof in the human body has been reduced so that they are useful as the active ingredient of the therapeutic agents of the present invention.

A preferred embodiment of the humanized antibody for use in the present invention includes humanized PM-1 antibody (see International Patent Application WO 92-19759).

1-7. Expression and production

Antibody genes constructed as described above may be expressed and obtained in a known method. In the case of mammalian cells, expression may be accomplished using a DNA in which a commonly used useful promoter, the antibody gene to be expressed, and the poly A signal at 3' downstream thereof have been operably linked or a vector containing said DNA. Examples of the promoter/enhancer include human cytomegalovirus immediate early promoter/enhancer.

Additionally, as the promoter/enhancer which can be used for expression of antibody for use in the present invention, there are viral promoters/enhancers such as retrovirus, polyoma virus, adenovirus, and simian virus 40 (SV40), and promoters/enhancers derived from mammalian cells such as human elongation factor 1 α (HEF1 α).

For example, expression may be readily accomplished by the method of Mulligan et al. (Nature

(1979) 277, 108) when SV40 promoter/enhancer is used, or by the method of Mizushima et al. (Nucleic Acids Res. (1990) 18, 5322) when HEF1 α promoter/enhancer is used.

5 In the case of E. coli, expression may be conducted by operably linking a commonly used useful promoter, a signal sequence for antibody secretion, and the antibody gene to be expressed, followed by expression thereof. As the promoter, for example, there can be mentioned lacz promoter and araB promoter. The method of
10 Ward et al. (Nature (1098) 341, 544-546; FASEB J. (1992) 6, 2422-2427) may be used when lacz promoter is used, and the method of Better et al. (Science (1988) 240, 1041-1043) may be used when araB promoter is used.

15 As the signal sequence for antibody secretion, when produced in the periplasm of E. coli, the pelB signal sequence (Lei, S. P. et al., J. Bacteriol. (1987) 169, 4379) can be used. After separating the antibody produced in the periplasm, the structure of the antibody is appropriately refolded before use (see, for example,
20 WO 96/30394).

As the origin of replication, there can be used those derived from SV40, polyoma virus, adenovirus, bovine papilloma virus (BPV) and the like. Furthermore, for the amplification of the gene copy number in the host
25 cell system, expression vectors can include as selectable markers the aminoglycoside transferase (APH) gene, the thymidine kinase (TK) gene, E. coli xanthine guaninephosphoribosyl transferase (Ecogpt) gene, the dihydrofolate reductase (dhfr) gene and the like.

30 For the production of antibody for use in the present invention, any production system can be used. The production system of antibody preparation comprises the in vitro or the in vivo production system. As the in vitro production system, there can be mentioned a
35 production system which employs eukaryotic cells and the production system which employs prokaryotic cells.

When the eukaryotic cells are used, there are

the production systems which employ animal cells, plant cells, and fungal cells. Known animal cells include (1) mammalian cells such as CHO cells, COS cells, myeloma cells, baby hamster kidney (BHK) cells, HeLa cells, and Vero cells, (2) amphibian cells such as Xenopus oocytes, or (3) insect cells such as sf9, sf21, and Tn5. Known plant cells include, for example, those derived from Nicotiana tabacum, which is subjected to callus culture. Known fungal cells include yeasts such as the genus Saccharomyces, more specifically Saccharomyces cerevisiae, or filamentous fungi such as the genus Aspergillus, more specifically Aspergillus niger.

When the prokaryotic cells are used, there are the production systems which employ bacterial cells. Known bacterial cells include Escherichia coli (E. coli), and Bacillus subtilis.

By introducing via transformation the gene of the desired antibody into these cells and culturing the transformed cells in vitro, the antibody can be obtained. Culturing is conducted in the known methods. For example, as the culture liquid, DMEM, MEM, RPMI1640, and IMDM can be used, and serum supplements such as fetal calf serum (FCS) may be used in combination. In addition, antibodies may be produced in vivo by implanting cells into which the antibody gene has been introduced into the abdominal cavity of an animal and the like.

As in vivo production systems, there can be mentioned those which employ animals and those which employ plants. When animals are used, there are the production systems which employ mammals and insects.

As mammals, goats, pigs, sheep, mice, and cattle can be used (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). Also, as insects, silkworms can be used.

When plants are used, tobacco, for example, can be used.

Antibody genes are introduced into these animals or plants, and the antibodies are produced in such animals or plants, and recovered. For example, an antibody gene is inserted into the middle of the gene encoding protein which is inherently produced in the milk such as goat β casein to prepare fusion genes. DNA fragments containing the fusion gene into which the antibody gene has been inserted are injected into a goat embryo, and the embryo is introduced into a female goat. The desired antibody is obtained from the milk produced by the transgenic goat born to the goat who received the embryo or offsprings thereof. In order to increase the amount of milk containing the desired antibody produced by the transgenic goat, hormones may be given to the transgenic goat as appropriate. (Ebert, K.M. et al., Bio/Technology (1994) 12, 699-702).

When silkworms are used, baculovirus into which the desired antibody gene has been inserted is infected to the silkworm, and the desired antibody can be obtained from the body fluid of the silkworm (Susumu, M. et al., Nature (1985) 315, 592-594). Moreover, when tobacco is used, the desired antibody gene is inserted into an expression vector for plants, for example pMON 530, and then the vector is introduced into a bacterium such as Agrobacterium tumefaciens. The bacterium is then infected to tobacco such as Nicotiana tabacum to obtain the desired antibody from the leaves of the tobacco (Julian, K.-C. Ma et al., Eur. J. Immunol. (1994) 24, 131-138).

When antibody is produced in in vitro or in vivo production systems, as described above, DNA encoding the heavy chain (H chain) or the light chain (L chain) of antibody may be separately integrated into an expression vector and the hosts are transformed simultaneously, or DNA encoding the H chain and the L chain may be integrated into a single expression vector and the host is transformed therewith (see International Patent

Application WO 94-11523).

1-8. Modified antibody

Antibodies for use in the present invention may be antibody fragments or modified versions thereof as long as they are preferably used. For example, as fragments of antibody, there may be mentioned Fab, F(ab')₂, Fv or single-chain Fv (scFv) in which Fv's of H chain and L chain were ligated via a suitable linker. Specifically antibodies are treated with an enzyme, for example, papain or pepsin, to produce antibody fragments, or genes encoding these antibody fragments are constructed, and then introduced into an expression vector, which is expressed in a suitable host cell (see, for example, Co, M. S. et al., J. Immunol. (1994) 152, 2968-2976; Better, M. and Horwitz, A.H., Methods in Enzymology (1989) 178, 476-496, Academic Press, Inc.; Plueckthun, A. and Skerra, A., Methods in Enzymology (1989) 178, 476-496, Academic Press, Inc.; Lamoyi, E., Methods in Enzymology (1986) 121, 652-663; Rousseaux, J. et al., Methods in Enzymology (1986) 121, 663-669; Bird, R.E. et al., TIBTECH (1991) 9, 132-137).

scFv can be obtained by ligating the V region of H chain and the V region of L chain of antibody. In the scFv, the V region of H chain and the V region of L chain are preferably ligated via a linker, preferably a peptide linker (Huston, J.S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883). The V region of H chain and the V region of L chain in the scFv may be derived from any of the above-mentioned antibodies. As the peptide linker for ligating the V regions, any single-chain peptide comprising, for example, 12 - 19 amino acid residues may be used.

DNA encoding scFv can be obtained using DNA encoding the H chain or the H chain V region of the above antibody and DNA encoding the L chain or the L chain V region of the above antibody as the template by amplifying the portion of the DNA encoding the desired

amino acid sequence among the above sequences by the PCR technique with the primer pair specifying the both ends thereof, and by further amplifying the combination of DNA encoding the peptide linker portion and the primer pair which defines that both ends of said DNA be ligated to the H chain and the L chain, respectively.

Once DNAs encoding scFv are constructed, an expression vector containing them and a host transformed with said expression vector can be obtained by the conventional methods, and scFv can be obtained using the resultant host by the conventional methods.

These antibody fragments can be produced by obtaining the gene thereof in a similar manner to that mentioned above and by allowing it to be expressed in a host. "Antibody" as used in the claim of the present application encompasses these antibody fragments.

As modified antibodies, antibodies associated with various molecules such as polyethylene glycol (PEG) can be used. "Antibody" as used in the claim of the present application encompasses these modified antibodies. These modified antibodies can be obtained by chemically modifying the antibodies thus obtained. These methods have already been established in the art.

1-9. Separation and purification of antibody
1-9-1. Separation and purification of antibody

Antibodies produced and expressed as described above can be separated from the inside or outside of the host cell and then may be purified to homogeneity. Separation and purification of the antibody for use in the present invention may be accomplished by affinity chromatography. As the column used for such affinity chromatography, there can be mentioned Protein A column and Protein G column. Examples of the carriers used in the Protein A column are Hyper D, POROS, Sepharose F. F. and the like. Alternatively, methods for separation and purification conventionally used for proteins can be used without any limitation. Separation and purification of

the antibody for use in the present invention may be accomplished by combining, as appropriate, chromatography other than the above-mentioned affinity chromatography, filtration, ultrafiltration, salting-out, dialysis and the like. Chromatography includes, for example, ion exchange chromatography, hydrophobic chromatography, gel-filtration and the like. These chromatographies can be applied into HPLC. Alternatively, reverse-phase chromatography can be used.

1-9-2. Determination of antibody concentration

The concentration of antibody obtained in the above 2-1 can be determined by the measurement of absorbance or by the enzyme-linked immunosorbent assay (ELISA) and the like. Thus, when absorbance measurement is employed, the antibody for use in the present invention or a sample containing the antibody is appropriately diluted with PBS(-) and then the absorbance is measured at 280 nm, followed by calculation using the absorption coefficient of 1.35 OD at 1 mg/ml. When the ELISA method is used, measurement is conducted as follows. Thus, 100 μ l of goat anti-human IgG (manufactured by TAGO) diluted to 1 μ g/ml in 0.1 M bicarbonate buffer, pH 9.6, is added to a 96-well plate (manufactured by Nunc), and is incubated overnight at 4 °C to immobilize the antibody.

After blocking, 100 μ l each of appropriately diluted antibody for use in the present invention or a sample containing the antibody, or 100 μ l of human IgG (manufactured by CAPPEL) as the standard is added, and incubated at room temperature for 1 hour. After washing, 100 μ l of 5000-fold diluted alkaline phosphatase-labeled anti-human IgG antibody (manufactured by BIO SOURCE) is added, and incubated at room temperature for 1 hour. After washing, the substrate solution is added and incubated, followed by the measurement of absorbance at

405 nm using the MICROPLATE READER Model 3550 (manufactured by Bio-Rad) to calculate the concentration of the desired antibody.

1-10. IL-6 antagonists other than antibodies

5 The altered IL-6 for use in the present invention has an activity of binding to IL-6 receptor and does not transmit the biological activity of IL-6. Thus, the altered IL-6, though it competes with IL-6 for binding to IL-6 receptor, does not transmit the biological activity of IL-6, and thereby it blocks signal transduction by IL-6.

 Altered IL-6 may be constructed through the introduction of mutation by replacing amino acid residues of the amino acid sequence of IL-6. IL-6, the source of the altered IL-6, may be of any origin, but when the antigenicity is to be considered, it is preferably human IL-6. Specifically, the secondary structure of IL-6 is predicted using a known molecular modeling program of the amino acid sequence, for example WHATIF (Vriend et al., J. Mol. Graphics (1990), 8, 52-56), and the overall effects of the amino acid residue to be replaced is evaluated. After an appropriate amino acid residue was determined, mutation is introduced by the commonly used polymerase chain reaction (PCR) method using a vector containing the base sequence encoding human IL-6 gene thereby to obtain a gene encoding altered IL-6. This is then integrated, as desired, into an appropriate expression vector, from which altered IL-6 can be obtained according to the expression, production and purification of said recombinant antibody.

 Specific examples of the altered IL-6 are disclosed in Brakenhoff et al., J. Biol. Chem. (1994) 269, 86-93, and Savino et al., EMBO J. (1994) 13, 1357-1367, WO 96-18648, and WO 96-17869.

35 The IL-6 partial peptide or the IL-6 receptor partial peptide for use in the present invention has an activity of binding to IL-6 receptor or IL-6,

respectively, and does not transmit the biological activity of IL-6. Thus, the IL-6 partial peptide or the IL-6 receptor partial peptide binds to IL-6 receptor or IL-6, respectively, and thereby capture it. As a result, they do not transmit the biological activity of IL-6, and block signal transduction of IL-6.

The IL-6 partial peptide or the IL-6 receptor partial peptide is a peptide comprising some or all of the amino acid sequence of the region involved in the binding to IL-6 and IL-6 receptor in the amino acid sequence of IL-6 or IL-6 receptor. Such a peptide generally comprises 10 - 80, preferably 20 - 50, more preferably 20 - 40 amino acid residues.

The IL-6 partial peptide or the IL-6 receptor partial peptide can be constructed by specifying the region involved in the binding to IL-6 and IL-6 receptor in the amino acid sequence of IL-6 or IL-6 receptor, and by producing some or all of the amino acid sequence by a conventional method such as a genetic engineering technology or a peptide synthesis method.

In order to prepare the IL-6 partial peptide or the IL-6 receptor partial peptide by a genetic engineering technology, the DNA sequence encoding the desired peptide is integrated into an expression vector, from which the peptide can be obtained according to the expression, production, and purification of said recombinant antibody.

Preparation of the IL-6 partial peptide or the IL-6 receptor partial peptide by the peptide synthesis method can be effected using a method commonly used in peptide synthesis such as the solid phase synthesis or the liquid phase synthesis. Specifically the method described in Zoku-iyakuhin no Kaihatsu (Sequel to Development of Pharmaceuticals), Vol. 14, Peptido Gousei (Peptide Synthesis), edited by Haruaki Yajima, Hirokawa Shoten, 1991, may be used. The solid phase synthesis method used includes, for example, a reaction in which an

amino acid corresponding to the C-terminal of the peptide to be synthesized is coupled to a support which is insoluble in organic solvents, and then an amino acid in which α -amino group or a side chain functional group has been protected with an appropriate protecting group is condensed one amino acid at a time from the C-terminal to the N-terminal direction, and a reaction in which said protecting group of the α -amino group of the amino acid or the peptide coupled to the resin is eliminated is alternately repeated to elongate the peptide chain. The solid phase peptide synthesis methods are divided into the Boc method and the Fmoc method depending on the type of protecting group to be used.

After the synthesis of the desired peptide is complete, the peptide chain is cleaved from the support via a deprotection reaction. For cleavage from the peptide chain, hydrogen fluoride or trifluoromethane sulfonate in the Boc method and TFA in the Fmoc method are generally used. In the Boc method, for example, the above peptide resin is treated in hydrogen fluoride in the presence of anisole. Subsequently, the protecting group is eliminated and the peptide is recovered by cleaving from the support. By lyophilizing this, crude peptide can be obtained. On the other hand, in the Fmoc method, TFA, for example, is used in a manner similar to the above to effect the deprotection reaction and the cleavage reaction of the peptide from the support.

The crude peptide thus obtained can be applied to HPLC for its separation and purification. Its elution can be carried out in a water-acetonitrile solvent system that is commonly used for protein purification under an optimum condition. The fraction corresponding to the peak of the profile of the chromatography obtained is collected and lyophilized. The peptide fraction thus purified is identified by subjecting it to the analysis of molecular weight by mass spectroscopic analysis, the

analysis of amino acid composition, or the analysis of amino acid sequence, and the like..

Specific examples of the IL-6 partial peptide or the IL-6 receptor partial peptide are disclosed in
5 Japanese Unexamined Patent Publication (Kokai) 2(1990)-188600, Japanese Unexamined Patent Publication (Kokai) 7(1995)-324097, Japanese Unexamined Patent Publication (Kokai) 8(1996)-311098, and United States Patent Publication US 5210075.

10 2. Confirmation of the activity of IL-6 antagonist

The activity of the IL-6 antagonist for use in the present invention can be evaluated using a conventionally known method. For example, IL-6 is added to the IL-6-dependent cell MH60.BSF2 and the activity can
15 be evaluated using the incorporation of ³H-thymidine into the IL-6-dependent cell in the coexistence of the IL-6 antagonist as an index. Alternatively, evaluation can be effected by adding ¹²⁵I-labeled IL-6 and an excess amount of non-labeled IL-6 to the U266, an IL-6 receptor-
20 expressing cell, and adding the IL-6 antagonist at the same time and then by determining the ¹²⁵I-labeled IL-6 bound to the IL-6 receptor-expressing cell.

3. Confirmation of therapeutic effects

In order to confirm the effects accomplished by the present invention, the IL-6 antagonist for use in the
25 present invention may be given to an animal that has been sensitized with T cells via challenge or to an animal to which sensitized T cells have been introduced, and the suppressive effects on the sensitized T cells are
30 evaluated.

As the sensitizing antigen to be given to the animal, for example, tubercle bacillus can be used.

As the animal to be immunized, animals generally used in the experiments can be used such as
35 mice, rats, rabbits, and the like. The effect of the present invention to be evaluated can be confirmed by observing the delayed inflammatory reaction induced by

challenging the same antigen to an animal that was given the antigen.

As described the examples below, in the mouse delayed foot pad edema reaction, the administration of anti-IL-6 receptor antibody resulted in suppression of the delayed inflammatory reaction was observed. Since it was known that sensitized T cells are involved in the delayed foot pad edema reaction, it was revealed that IL-6 antagonists such as anti-IL-6 receptor antibody exert an inhibitory effect on the sensitized T cells.

4. Route of administration and pharmaceutical preparation

The preventive or therapeutic agents for sensitized T cell-mediated diseases of the present invention may be administered, either systemically or locally, by a parenteral route, for example intravenous injection such as drip infusion, intramuscular injection, intraperitoneal injection, and subcutaneous injection. The method of administration may be chosen, as appropriate, depending on the age and the conditions of the patient. The effective dosage is chosen from the range of 0.01 mg to 100 mg per kg of body weight per administration. Alternatively, the dosage in the range of 1 to 1000 mg, preferably 5 to 50 mg per patient may be chosen.

The preventive or therapeutic agents for sensitized T cell-mediated diseases of the present invention may contain pharmaceutically acceptable carriers or additives depending on the route of administration. Examples of such carriers or additives include water, a pharmaceutical acceptable organic solvent, collagen, polyvinyl alcohol, polyvinylpyrrolidone, a carboxyvinyl polymer, carboxymethylcellulose sodium, polyacrylic sodium, sodium alginate, water-soluble dextran, carboxymethyl starch sodium, pectin, methyl cellulose, ethyl cellulose, xanthan gum, gum Arabic, casein, gelatin, agar,

diglycerin, propylene glycol, polyethylene glycol, Vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose, a pharmaceutically acceptable surfactant and the like.

5 Additives used are chosen from, but not limited to, the above or combinations thereof depending on the dosage form.

The subject diseases to be prevented or treated of the present invention are diseases in which sensitized
10 T cell are involved. Specifically, they include delayed hypersensitivity, chronic thyroiditis, uveitis, atopic dermatitis, contact dermatitis, or multiple sclerosis. The preventive or therapeutic agents of the present invention are useful as preventive or therapeutic agents
15 for diseases in which these sensitized T cells are involved.

Examples

The present invention will now be explained in more details with reference to the working examples, reference
20 examples, and experimental examples. It should be noted, however, that the present invention is not limited to them in any way.

Example 1. Inhibitory effects on delayed foot pad reaction

25 Dry dead cells of Mycobacterium butyricum were added to 2.5 mg/ml in the Freund's incomplete adjuvant to prepare an emulsion, 0.2 ml of which was then subcutaneously injected to C57BL/6 male mice for
30 challenge. On day 14, 10 mg of dry dead cells of Mycobacterium butyricum suspended in physiological saline were subcutaneously injected to the right foot pad of the animal to evoke the reaction. Twenty four hours later, the weights of the left and the right foot pad were
35 measured and the difference in the weights were used as an index of the strength of the reaction.

MR16-1 antibody at 0.125 mg, 0.5 mg, or 2 mg was

intraperitoneally given only once simultaneously with the challenge. The control group received rat IgG (KH-5) having the same isotype and the unsensitized mouse group received physiological saline in a similar manner. The result is shown in Figure 1.

The one time administration of MR16-1 antibody on the day of challenge inhibited the delayed foot pad edema reaction in a dose-dependent manner.

Reference example 1. Preparation of human soluble IL-6 receptor

Soluble IL-6 receptor was prepared by the PCR method using a plasmid pBSF2R.236 containing cDNA that encodes IL-6 receptor obtained according to the method of Yamasaki et al., Science (1988) 241, 825-828. Plasmid pBSF2R.236 was digested with a restriction enzyme Sph I to obtain the cDNA of IL-6 receptor, which was then inserted into mpl8 (manufactured by Amersham). Using a synthetic oligoprimers designed to introduce a stop codon into the cDNA of IL-6 receptor, a mutation was introduced into the cDNA of IL-6 receptor by the PCR method using the in vitro Mutagenesis System (manufactured by Amersham). The procedure resulted in the introduction of a stop codon to the amino acid at position 345, and gave the cDNA encoding soluble IL-6 receptor.

In order to express the cDNA of soluble IL-6 receptor in CHO cells, it was ligated to plasmid pSV (manufactured by Pharmacia) to obtain plasmid pSVL344. The cDNA of soluble IL-6 receptor that was cleaved with Hind III-Sal I was inserted to plasmid pCEEdhfr containing the cDNA of dhfr to obtain plasmid pCEEdhfr344 that can be expressed in the CHO cells.

Ten µg of plasmid pCEEdhfr344 was transfected to a dhfr-CHO cell line DXB-11 (Urland et al., Proc. Natl. Acad. Sci. U.S.A. (1980) 77, 4216-4220) by the calcium phosphate method (Chen et al., Mol. Cell. Biol. (1987) 7, 2745-2751). The transfected CHO cells were cultured in a

nucleoside-free α MEM selection medium containing 1 mM glutamin, 10% dialyzed FCS, 100 U/ml penicillin, and 100 μ g/ml streptomycin.

5 The selected CHO cells were screened by the limiting dilution method to obtain a single CHO cell clone. The CHO cell clone was amplified in 20 nM to 200 nM methotrexate (MTX) to obtain a CHO cell line 5E27 that produces human soluble IL-6 receptor. The CHO cell line 5E27 was cultured in an Iscov-modified Dulbecco's medium
10 (IMDM, manufactured by Gibco) containing 5% FBS. The culture supernatant was collected and the concentration of soluble IL-6 receptor in the culture supernatant was determined by ELISA.

15 Reference example 2. Preparation of anti-human IL-6 antibody

Ten μ g of the recombinant IL-6 (Hirano et al., Immunol. Lett., 17:41, 1988) was immunized to BALB/c mice together with Freund's complete adjuvant, and this was repeated every week until anti-IL-6 antibody could be
20 detected in the serum. Immune cells were extracted from local lymph node and were then fused with a myeloma cell line P3U1 using polyethylene glycol 1500. Hybridomas were selected according to the method of Oi et al. (Selective Methods in Cellular Immunolgy, W. H. Freeman and Co., San Francisco, 351, 1980) that employs the HAT
25 medium, and the hybridoma that produces anti-human IL-6 antibody was established.

The hybridoma that produces anti-human IL-6 antibody was subjected to IL-6 binding assay as follows. Thus, a
30 96-well microtiter plate made of flexible polyvinyl (manufactured by Dynatech Laboratories, Inc., Alexandria, VA) was coated with 100 μ l of goat anti-mouse Ig (10 μ l/ml, manufactured by Cooper Biomedical, Inc., Malvern, PA) in 0.1 M carbonate-hydrogen carbonate buffer, pH9.6, overnight at 4 $^{\circ}$ C. Subsequently, the plate was treated
35

with PBS containing 1% bovine serum albumin (BSA) at room temperature for 2 hours.

After washing in PBS, 100 μ l of the hybridoma culture supernatant was added to each well, and then was incubated overnight at 4 °C. The plate was washed, 125 I-labelled recombinant IL-6 was added to each well to a concentration of 2000 cpm/0.5 ng/well, and then radioactivity of each well after washing was determined by a gamma counter (Beckman Gamma 9000, Beckman Instruments, Fullerton, CA). Of 216 hybridoma clones, 32 were positive in the IL-6 binding assay. From these clones, stable MH166.BSF2 was finally obtained. Anti-IL-6 antibody MH166 produced by said hybridoma has a subtype of IgG1 κ .

Then, the IL-6-dependent mouse hybridoma clone MH166.BSF2 was used to examine a neutralizing activity with respect to the growth of the hybridoma by MH166. MH166.BSF2 cells were aliquoted to 1×10^4 /200 μ l/well, and samples containing MH166 antibody were added thereto, cultured for 48 hours, 15.1 Ci/mM 3 H-thymidine (New England Nuclear, Boston, MA) was added, and the culturing was continued for further 6 hours. The cells were placed on a glass filter paper and were treated by the automatic harvester (Labo Mash Science Co., Tokyo, Japan). As the control, rabbit anti-IL-6 antibody was used.

As a result, MH166 antibody inhibited the incorporation of 3 H-thymidine of MH166.BSF2 cells induced by IL-6 in a dose dependent manner. This revealed that MH166 antibody neutralizes the activity of IL-6.

Reference example 3. Preparation of anti-human IL-6 receptor antibody

Anti-IL-6 receptor antibody MT18 prepared by the method of Hirata et al. (J. Immunol., 143, 2900-2906, 1989) was bound to CNBr-activated Sepharose 4B (manufactured by Pharmacia Fine Chemicals, Piscataway,

NJ) according to the attached regimen and IL-6 receptor (Science (1988) 241, 825-828) was purified. A human myeloma cell line U266 was solubilized with 1 mM p-para-aminophenyl methane sulphonyl fluoride hydrochloride (manufactured by Wako Chemicals) containing 1% digitonin (manufactured by Wako Chemicals), 10 mM triethanolamine (pH 7.8) and 0.15 M NaCl (digitonin buffer), and mixed with MT18 antibody bound to Sepharose 4B beads. Then, the beads were washed six times with the digitonin buffer, to prepare the partially purified IL-6 receptor.

BALB/c mice were immunized four times every ten days with the above partially purified IL-6 receptor obtained from 3×10^9 U266 cells, and then a hybridoma was prepared using a standard method. The hybridoma culture supernatant from the growth-positive well was tested for its activity of binding to IL-6 receptor according to the method described below. 5×10^7 U266 cells were labeled with ^{35}S -methionine (2.5 mCi) and were solubilized with the above digitonin buffer. The solubilized U266 cells were mixed with a 0.04 ml volume of MT18 antibody bound to Sepharose 4B beads, and then were washed six times with the digitonin buffer. ^{35}S -methionine-labeled IL-6 receptor was eluted with 0.25 ml of the digitonin buffer (pH 3.4) and was neutralized in 0.025 ml of 1M Tris (pH 7.4).

0.05 ml of the hybridoma culture supernatant was mixed with 0.01ml of Protein G Sepharose (manufactured by Pharmacia). After washing, Sepharose was incubated with 0.005 ml ^{35}S -labeled IL-6 receptor solution prepared as described above. The immunoprecipitate was analyzed by SDS-PAGE to investigate the hybridoma culture supernatant that reacts with IL-6 receptor. As a result, the reaction positive hybridoma clone PM-1 was established. The antibody produced from the hybridoma PM-1 has a subtype of IgG1k.

The inhibitory activity of IL-6 binding of the antibody produced by the hybridoma PM-1 to human IL-6

receptor was studied using the human myeloma cell line U266. A human recombinant IL-6 was prepared from E. coli (Hirano et al., Immunol. Lett., 17:41, 1988), and was labeled with ^{125}I using the Bolton-Hunter reagent (New England Nuclear, Boston, MA) (Taga, T. et al., J. Exp. Med. (1987) 166, 967). 4 x 10^5 U266 cells were cultured with the 70% (v/v) culture supernatant of hybridoma PM-1 together with 14,000 cpm of ^{125}I -labeled IL-6 in the presence of 100-fold excess of unlabeled IL-6 for one hour at room temperature. Seventy μl of the sample was layered on 300 μl FCS in a 400 μl microfuge polyethylene tube. After centrifugation, the radioactivity of the cell was determined.

The result revealed that the antibody produced by the hybridoma PM-1 inhibits the binding of IL-6 to IL-6 receptor.

Reference example 4. Preparation of anti-mouse IL-6 receptor antibody

A monoclonal antibody directed against mouse IL-6 receptor was prepared according to the method described in Saito, et al., J. Immunol. (1993) 147, 168-173.

The CHO cells that produce mouse soluble IL-6 receptor were cultured in the IMDM liquid medium containing 10% FCS. From the culture supernatant, mouse soluble IL-6 receptor was purified using mouse soluble IL-6 receptor RS12 (see Saito, et al., supra) and an affinity column fixed to Affigel 10 gel (Biorad).

The mouse soluble IL-6 receptor (50 μg) thus obtained was mixed with Freund's complete adjuvant, which was then injected to the abdomen of Wistar rats (Japan Charles River). From 2 weeks the animals were boosted with Freund's incomplete adjuvant. On day 45, the rats were sacrificed, and the spleen cells at about 2×10^8 were fused with 1×10^7 mouse myeloma cells P3U1 using 50% PEG1500 (Boehringer Mannheim) according to the conventional method, and then were screened by the HAT

culture medium.

After the culture supernatant was added to the plate coated with rabbit anti-rat IgG antibody (Cappel), mouse soluble IL-6 receptor was reacted. Subsequently, using rabbit anti-mouse IL-6 receptor antibody and alkaline phosphatase-labeled sheep anti-rabbit IgG, hybridomas producing antibody directed against mouse soluble IL-6 receptor were screened by ELISA. After antibody production was confirmed, the hybridoma clones were subscreened twice to obtain a single hybridoma clone. The clone was designated as MR16-1.

The neutralizing activity of the antibody produced by the hybridoma on signal transduction of mouse IL-6 was examined by ^3H -thymidine incorporation using MH60.BSF2 cells (Matsuda et al., J. Immunol. (1988) 18, 951-956). To a 96-well plate, MH60.BSF2 cells were prepared at 1×10^4 cells/200 μl /well. To the plate were added mouse IL-6 and MR16-1 antibody or RS12 antibody at 12.3 - 1000 ng/ml, and then were cultured at 37°C and 5% CO_2 for 44 hours and then 1 μCi /well of ^3H -thymidine was added. After 4 hours, the incorporation of ^3H -thymidine was measured. As a result, MR16-1 antibody suppressed the incorporation of ^3H -thymidine of the MH60.BSF2 cells.

Thus, it was demonstrated that the antibody produced by the hybridoma MR16-1 inhibits the binding of IL-6 to IL-6 receptor.

Industrial Applicability

In accordance with the present invention, it was shown that IL-6 antagonists such as anti-IL-6 receptor antibody has a suppressive effect on the sensitized T cells. Thus, it was indicated that IL-6 antagonists are useful as a therapeutic agent for multiple sclerosis, uveitis, chronic thyroiditis, delayed hypersensitivity, contact dermatitis, or atopic dermatitis.

Reference to the microorganisms deposited under the

Patent Cooperation Treaty, Rule 13-2, and the name of the
Depository institute

Depository institute

5 Name: the National Institute of Bioscience and Human
 Technology, Agency of Industrial Science and
 Technology

Address: 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki,
 Japan

Microorganism (1)

10 Indication: Rat-mouse hybridoma MR16-1
 Deposition number: FERM BP-5875
 Deposition date: March 13, 1997

Microorganism (2)

 Indication: HB 101-pIBIBSF2R
15 Deposition number: FERM BP-2232
 Deposition date: January 9, 1989

Microorganism (3)

 Indication: PM1
 Deposition number: FERM BP-2998
20 Deposition date: July 12, 1989

Depository organ

Name: National Collection of Industrial and Marine
 Bacteria Limited

Address: 23 St Machar Drive Aberdeen AB2 1RY

25 Microorganism (4)

 Indication: Escherichia coli DH5 α -pPM-k3
 Deposition number: NCIMB 40366
 Deposition date: February 12, 1991

Microorganism (5)

30 Indication: Escherichia coli DH5 α -pPM-h1
 Deposition number: NCIMB 40362
 Deposition date: February 12, 1991

CLAIMS

1. A preventive or therapeutic agent for sensitized T cell-mediated diseases comprising an interleukin-6 (IL-6) antagonist as an active ingredient.

5 2. The preventive or therapeutic agent according to claim 1, wherein the IL-6 antagonist is an antibody directed against IL-6 receptor.

3. The preventive or therapeutic agent according to claim 2, wherein the IL-6 antagonist is a monoclonal antibody directed against IL-6 receptor.

10 4. The preventive or therapeutic agent according to claim 3, wherein the IL-6 antagonist is a monoclonal antibody directed against human IL-6 receptor.

5. The preventive or therapeutic agent according to claim 3, wherein the IL-6 antagonist is a monoclonal antibody directed against mouse IL-6 receptor.

6. The preventive or therapeutic agent according to claim 4, wherein the IL-6 antagonist is PM-1 antibody.

20 7. The preventive or therapeutic agent according to claim 5, wherein the IL-6 antagonist is MR16-1 antibody.

8. The preventive or therapeutic agent according to claim 4, wherein the IL-6 antagonist is an antibody directed against IL-6 receptor having the constant region of human antibody.

9. The preventive or therapeutic agent according to claim 4, wherein the IL-6 antagonist is a chimeric or humanized antibody directed against IL-6 receptor.

30 10. The preventive or therapeutic agent according to claim 9, wherein the IL-6 antagonist is humanized PM-1 antibody.

11. The preventive or therapeutic agent according to any of claims 1 to 10, wherein the sensitized T cell-mediated diseases are multiple sclerosis, uveitis, chronic thyroiditis, delayed hypersensitivity, contact dermatitis, or atopic dermatitis.

12. A sensitized T cell suppressive agent

comprising an IL-6 antagonist as an active ingredient.

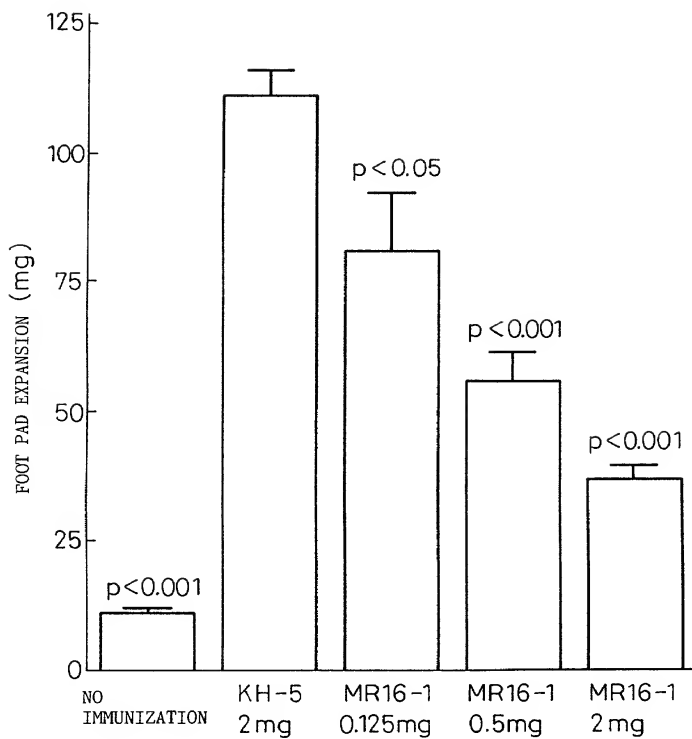
13. A sensitized T cell suppressive agent comprising antibody directed against IL-6 receptor as an active ingredient.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217
2218
2219
2220
2221
2222
2223
2224
2225
2226
2227

ABSTRACT

5 A preventive or therapeutic agent for sensitized T
cell-mediated diseases comprising an interleukin-6 (IL-6)
antagonist, for example an antibody directed against IL-6
receptor, an antibody directed against IL-6, an antibody
directed against gp130, and the like.

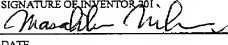
Fig.1



DECLARATION FOR PATENT APPLICATION (Includes Reference to PCT International Applications)			ATTORNEY'S DOCKET NUMBER <div style="text-align: center;">350292000800</div>
<p>As a below named inventor I hereby declare that:</p> <p>My residence, post office address and citizenship are as stated below next to my name,</p> <p>I believe I (am-) the original, first and sole/joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:</p> <p style="margin-left: 40px;">A PREVENTIVE OR THERAPEUTIC AGENT FOR SENSITIZED T CELL-MEDIATED DISEASES COMPRISING IL-6 ANTAGONIST AS AN ACTIVE INGREDIENT</p> <p>the specification of which (check only one item below):</p> <div style="margin-left: 40px;"> <input type="checkbox"/> is attached hereto. <input type="checkbox"/> was filed as United States application <div style="margin-left: 40px;"> Serial No. on and was amended on (if applicable). </div> <input checked="" type="checkbox"/> was filed as PCT international application <div style="margin-left: 40px;"> Number PCT/JP98/01217 ✓ on March 20, 1998 ✓ and was amended under PCT Article 19 on (if applicable). </div> </div> <p>I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.</p> <p>I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37 Code of Federal Regulations § 1.56(a) and (b).</p> <p>I hereby claim foreign priority benefits under Title 35 United States Code § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:</p>			
PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119:			
COUNTRY (if PCT indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. § 119
Japan	9-68467- (Pat. Appln.)	21, March, 1997	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

205	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
206	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
207	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
208	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
209	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
210	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
211	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
212	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE August 26, 1999	DATE	DATE
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE
SIGNATURE OF INVENTOR 207	SIGNATURE OF INVENTOR 208	SIGNATURE OF INVENTOR 209
DATE	DATE	DATE
SIGNATURE OF INVENTOR 210	SIGNATURE OF INVENTOR 211	SIGNATURE OF INVENTOR 212
DATE	DATE	DATE

Declaration for Patent Application (Continued) (Includes Reference to PCT International Applications)			ATTORNEY'S DOCKET NUMBER		
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.					
PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. § 120:					
U.S. APPLICATIONS			STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.			STATUS (Check one)		
PCT APPLICATION NUMBER	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)	PATENTED	PENDING	ABANDONED
POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)					
Thomas E. Ciotti (Reg No. 21,013), Kate H. Murashige (Reg No. 29,959), Gladys H. Monroy (Reg No. 32,430), Debra Shetka (Reg No. 33,309), Paul Schenck (Reg No. 27,253), E. Thomas Wheelock (Reg No. 28,825), Freddie K. Park (Reg No. 35,636), Susan K. Lehnhardt (Reg No. 33,943), Patricia M. Drost (Reg No. 29,790), Shmuel Livnat (Reg No. 33,949), Tyler Dylan (Reg No. 37,612), Reid G. Adler (Reg No. 30,988), Gary A. Green (Reg No. 38,474), Antoinette F. Konski (Reg No. 34,202), Harry J. Macey (Reg No. 32,818), Stuart P. Kaler (Reg No. 35,913), David L. Bradfute (Reg No. 39,117), Robert Saltzberg (Reg No. 36,910), Laurie A. Axford (Reg No. 35,053), Mani Adeli (Reg No. 39,585), Catherine M. Polizzi (Reg No. 40,130), Sean Brennan (Reg No. 39,917), James C. Peacock III (Reg No. 40,124), J. Michael Schiff (Reg No. 40,253), Robert A. Millman (Reg No. 36,217), Robert K. Cerpa (Reg No. 39,933), Ronald D. Devore (Reg No. 39,958), Lee K. Tan (Reg No. 39,447)					
Send correspondence to: Kate H. Murashige Morrison & Foerster LLP 2000 Pennsylvania Ave., NW, Ste. 5500 Washington, DC 20006-1888			Direct telephone calls to: Kate H. Murashige at (202) 887-1533		
201	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
		Mihara	Masahiko		
	RESIDENCE & CITIZENSHIP	CITY		STATE OR FOREIGN COUNTRY	
		Gotenba-shi, Shizuoka, Japan		Japan	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS			
		C/O CHUGAI SEIYAKU KABUSHIKI KAISHA, 135, Kohnakado 1-chome,			
		Gotenba-shi, Shizuoka, Japan			
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY		STATE OR FOREIGN COUNTRY	
	POST OFFICE ADDRESS	CITY			
		STATE & ZIP CODE/COUNTRY			
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY		STATE OR FOREIGN COUNTRY	
	POST OFFICE ADDRESS	CITY			
		STATE & ZIP CODE/COUNTRY			
204	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY		STATE OR FOREIGN COUNTRY	
	POST OFFICE ADDRESS	CITY			
		STATE & ZIP CODE/COUNTRY			